BMC Cancer

RESEARCH

Open Access

# MEF2A, MEF2C, and MEF2D as potential biomarkers of pancreatic cancer?

Chunxia Zhai[1*†], Xiaorong Ding[1†], Liping Mao[2†], Yang Ge[1], Anqi Huang[1], Fan Yang[1] and Yi Ding[1*]

## Abstract

**Background** The myocyte enhancer factor-2 (MEF2) family genes were involved in the carcinogenesis and prognosis of multiple human tumors. The impact of MEF2s on the occurrences, progression, and clinical outcome of pancreatic cancer (PAAD) remains unknown.

**Methods** This study used the CCLE, HPA, EMBL-EBI, and GEPIA2 databases to study MEF2s expression in PAAD patients. We also investigated the relationship between MEF2s expression and methylation through the DiseaseMeth database, and used MEXPRESS to verify the association. Then we utilized the Kaplan–Meier Plotter and GEPIA2 databases to evaluate the prognostic value of MEF2s in PAAD. The cBioPortal database was used to explore the alteration features of MEF2s in PAAD. We then investigated the association between MEF2s expression, immune cells infiltration, and immune infiltration markers using the TIMER database. Finally, Metascape, STRING, and Cytoscape tools were used for functional enrichment analysis.

**Results** MEF2A, MEF2C, and MEF2D were found to be highly expressed in PAAD patients' tissues compared to normal tissues, whereas MEF2B expression did not show significant differential expression. In addition, the protein expression of MEF2A, MEF2C, and MEF2D was higher in PAAD tissues. Negative correlations were observed between the expression level of MEF2A, MEF2C, and MEF2D and the methylation levels in multiple sites. High expression of MEF2A was related to poor overall survival ($p=0.0071$) and relapse-free survival (RFS) ($p=0.0089$) of PAAD. High expression of MEF2C was associated with worse RFS of PAAD ($p=0.043$). MEF2A was a Truncating mutation, and it was shown that the "G27Wfs*8" mutation point was distributed in the SRF-TF domain. Both MEF2C and MEF2D were a Missense mutation. MEF2A, MEF2C, and MEF2D expression was positively corresponded with five immune cells infiltration (CD8 + T cells, B-cells, neutrophils, macrophages, and dendritic cells), especially for CD8 + T cells and macrophages. Among the 20 pathways, hsa05140 (Leishmania infection), hsa04022 (cGMP-PKG signaling pathway), hsa05145 (Toxoplasmosis), hsa04371 (Apelin signaling pathway), and hsa04064 (NF-kappa B signaling pathway), were closely connected with the occurrence and development of PAAD.

**Conclusions** Our results indicated that the overexpression of MEF2A, MEF2C, and MEF2D in patients with PAAD. MEF2A could be used as a prognostic biomarker for PAAD, MEF2C might be a potential oncogene for PAAD, and MEF2D had potential biological significance.

†Chunxia Zhai, Xiaorong Ding, and Liping Mao contributed equally to this work.

*Correspondence:
Chunxia Zhai
zhaichunxia@yeah.net
Yi Ding
ntdingyi@126.com
Full list of author information is available at the end of the article

**Keywords** MEF2A, MEF2C, MEF2D, Pancreatic cancer

## Introduction

Globally, pancreatic cancer (PAAD) ranks 13 th among the most common carcinomas, and it is also the 7 th most common reason for death in patients with cancer [1]. According to Rahib et al [2]., PAAD would become the second most deadly cancer by 2030. The five-year survival of PAAD is only 7.7%, with the median overall survival time less than one year [3]. Although surgery and drugs improve the prognosis to a certain extent, the mortality rate of PAAD is still increasing at an annual rate of 0.3% [4]. In addition, the low registration rate of clinical trials in PAAD reduces the development of new therapies [5]. It is worth noting that because the symptoms of patients with early-stage PAAD are not obvious, nearly 80–85% of the cases have been unresectable advanced or metastatic disease at the initial diagnosis, and their median survival time is only 3–14 months [6]. Therefore, screening effective biomarkers for the diagnosis, treatment, and prognostic value of PAAD patients is of important clinical significance.

The myocyte enhancer factor- 2 (MEF2) family has four members: MEF2A, MEF2B, MEF2C, and MEF2D, which are originally confirmed as critical transcriptional activators in muscle development [7, 8]. Later, studies found that the MEF2 factors affect the nerve, heart, vessel evolution, and growth factor responsiveness [9–12]. Additionally, many studies reported that MEF2s are closely connected with the evolution of multiple tumors, such as, diffuse large B cell lymphoma [13], and several solid tumors (ovarian cancer [14], gallbladder cancer [15], and hepatocellular carcinoma [16]) are correlated with MEF2s. The focus of our research was to explore the multimolecular role of the MEF2s gene family in the carcinogenesis, development, and prognosis of PAAD from the aspect of gene expression, genetic alteration, DNA methylation, and immune cells infiltration.

## Materials & methods

### Expression analysis

We used CCLE (https://www.broadinstitute.org/ccle), HPA (https://www.proteinatlas.org/), and EMBL-EBI (https://www.ebi.ac.uk) databases to evaluate MEF2s expression in PAAD cell lines [17–19]. Using the GEPIA2 database (http://gepia.cancer-pku.cn/), we analyzed MEF2s expression in PAAD tissues and normal pancreatic tissues, while further correlation analysis of MEF2s expression was carried out [20]. The student's *t*-test was used to contrast the PAAD tissues and normal tissues. Filters settings: analysis type, pancreatic cancer vs. normal analysis; *P*-value, 0.05; fold change, all; gene rank, 1%. Datasets, genes, *P*-value, fold change, and *t*-test were chosen for statistically significant analyses. The investigation referred to the following studies of PAAD, including Badea Pancreas, TCGA Pancreas, and Logsdon Pancreas studies [21, 22]. In addition, we also used the HPA database to show the immunohistochemistry images of MEF2s protein expression in PAAD tissues and normal pancreatic tissues [17]. Furthermore, using UALCAN (http://ualcan.path.uab.edu) and TISIDB database (http://cis.hku.hk/TISIDB/index.php) to study the relationship between MEF2s expression and clinicopathological features (tumor grades and immune subtypes) in PAAD [23, 24]. The expression of MEF2s in PAAD was validated using an independent dataset (GSE62452) obtained from the GEO database (https://www.ncbi.nlm.nih.gov/geo/). The dataset comprised 69 tumor tissues and 61 normal controls.

### Methylation analysis

We tested the methylation profile of MEF2s family members in the UALCAN database [23].$P < 0.05$ was considered as statistically significant. At the same time, using the MEXPRESS database (https://mexpress.ugent.be/) [25], we further explored the association between MEF2s expression and methylation status in PAAD. DiseaseMeth (v3.0, http://diseasemeth.edbc.org/) serves as a comprehensive methylation data of database, consolidating extensive DNA methylation profiles obtained through microarray and next-generation sequencing technologies, with functional annotations for disease-associated methylation patterns [26]. This platform was used to compare the methylation levels of each hub gene between PAAD and normal tissues.

### Survival analysis

We used Kaplan–Meier Plotter (http://www.kmplot.com) and GEPIA2 databases to analyze over survival (OS), relapse-free survival (RFS), and disease-free survival (DFS) of MEF2s in PAAD [20, 27]. A total of 177 PAAD patients for OS, 69 PAAD patients for RFS, and 178 PAAD patients for DFS were analyzed, except for the MEF2B-related DFS analysis, which included 175 patients. The sample sizes of Immune cells were as follows: B cells ($n = 59$), CD4 + memory T cells ($n = 43$), CD8 + T cells ($n = 76$), eosinophils ($n = 154$), macrophages ($n = 109$), mesenchymal stem cells ($n = 156$), natural killer T cells ($n = 60$), and regulatory T-cells ($n = 67$). The selection of the "Auto select best cutoff" was

employed in the analytical [28]. Subsequently, the samples were categorized into high expression group and low expression group based on the determined cutoff. Subsequent to this classification, the hazard ratio (HR) along with 95% confidence intervals (CI) and log-rank *P* values were computed, with adjustments made for multiple comparisons via the false discovery rate. Univariate and Multivariate Cox regression models was utilized to determine the association between an array of clinical characteristics (including pathological stage, age, primary therapy outcome, and radiation therapy) and the expression levels of the MEF2s. Statistical significance was denoted by a *p*-value below 0.05. The outcomes of the Cox regression model were integrated with independent prognostic variables obtained from multivariate analysis, and these data were used to predict survival at 1, 2, and 3 years. A calibration curve was employed to assess the concordance between the predicted probability and the actual probability of occurrence, with the 45-degree line symbolizing the ideal predictive value.

#### Alteration analysis

We analyzed the alteration frequency and mutation types of MEF2s in PAAD by the cBioPortal database (http://www.cbioportal.org/) [29, 30]. The search parameters had mutations from GISTIC and putative copy-number alterations. Besides, we further studied the relationship between mutation and prognosis.

#### Immune analysis

We explored the association between immune infiltration and MEF2s expression in PAAD through the TIMER database (https://cistrome.shinyapps.io/timer/) [31]."Gene module"was used to investigate the association between MEF2s and immune cells infiltration. The association between MEF2s expression and multiple immune infiltration markers in PAAD was further evaluated by the"Correlation module". TBtools generated a heatmap of the correlation between MEF2s and infiltrating immune cells [32].

#### Enrichment analysis

We achieved the top 20 genes that were most relevant to the expression of the four members of the MEF2s family in PAAD by GEPIA2 database, and then added MEF2A-D itself, deleted duplicate genes, and finally 82 neighboring genes for further analysis. We then used the STRING database (http://string-db.org/) to provide proteins' prediction and experimental interaction information and construct a gene regulatory network [33]. Next, we outputted the results from the STRING database analysis to Cytoscape to visualize the gene regulatory network. Under the default parameters, we calculated the properties of the PPI network. We used the Molecular Complex Detection (MCODE) plug-in model in the Cytoscape to recognize closely connected modules. Furthermore, to predict the functional roles of target genes, we used the Metascape database (http://www.metascape.org/) to do GO enrichment analysis from three directions, including BP, CC, and MF [34]. At the same time, KEGG analysis was carried out to identify the pathways related to the functions of MEF2s alterations and the frequently altered adjacent genes. In addition, the design flow chart of this research was presented in Fig. 1.

## Results

#### The expression of MEF2s in PAAD cell lines

This study utilized several online databases to analyze the expression of MEF2s in PAAD patients. Using the CCLE database, we found statistically significant expression of MEF2A and MEF2D in PAAD cell lines compared to normal cell lines (Log2 (TPM + 1) > 1, $P < 0.05$) (Fig. 2A-D). In addition, MEF2A and MEF2D were abnormally expressed in PAAD cell lines compared with normal cell lines (MEF2A, AverageExpression = 20.3nTPM, and MEF2D, AverageExpression = 28.1nTPM) (Fig. 2E-H). This study also used the European Bioinformatics Institute (EMBL-EBI) database to evaluate further the MEF2s expression in PAAD cell lines compared with normal cell lines. As shown in Fig. 2I-L, MEF2A, MEF2C, and MEF2D were abnormally expressed in multiple samples.

#### Association of expression of MEF2s with PAAD in human cancers

As shown in Fig. 3A, MEF2A, MEF2C, and MEF2D were found to be highly expressed in PAAD patients' tissues compared to normal tissues, whereas MEF2B expression did not show significant differential expression between PAAD cases and normal samples. In addition, we further contrasted MEF2s expression between PAAD tissues and normal pancreatic tissues in terms of mRNA and protein by using the GEPIA2 database and the HPA databases (Fig. 3B-E). As presented in Fig. 3B, MEF2A had a higher degree of mRNA expression in PAAD tissues, and medium protein expression in PAAD tissues, low protein expression in normal pancreatic tissues. In PAAD tissues and normal pancreatic tissues, we noticed that MEF2B mRNA and protein were not expressed (Fig. 3C). MEF2C had a higher level of mRNA and protein expression in PAAD tissues (Fig. 3D). MEF2D had a higher degree of mRNA expression in PAAD tissues, the protein expression of MEF2D was high in both PAAD tissues and normal pancreatic tissues (Fig. 3E). Similarly, we verified the correlation between the expression of MEF2A, MEF2C, and MEF2D and copy number variation (CNV) was statistically significant through the CCLE database (Fig. 3F).

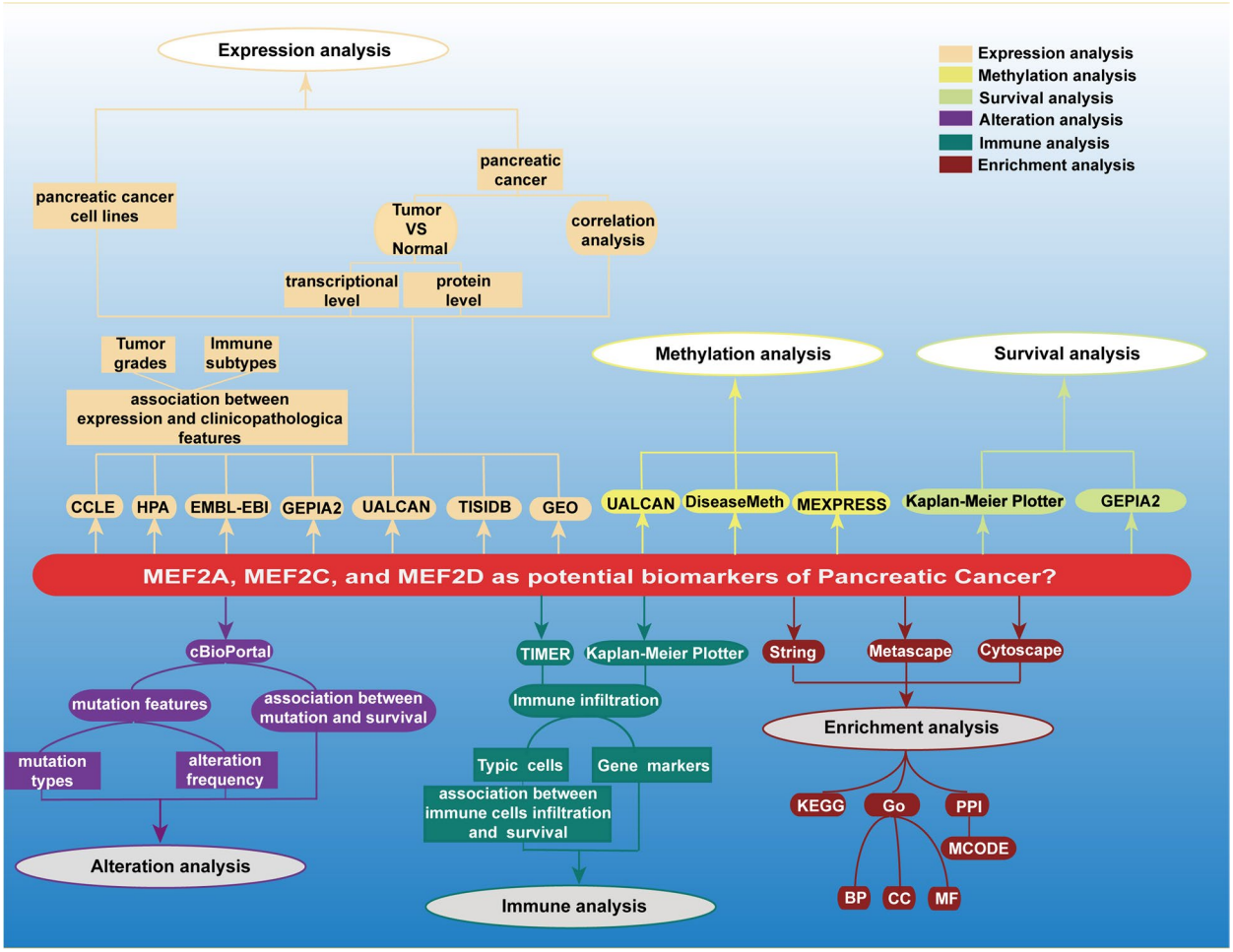


**Fig. 1** Design flow chart of this study

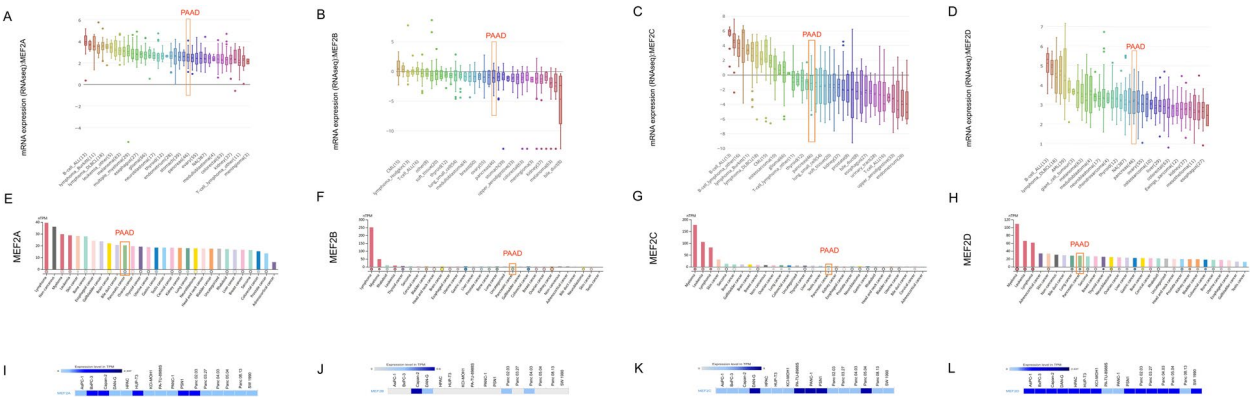


**Fig. 2** The expression of MEF2s in human cancer cell lines including PAAD cell lines. **A**–**D** Cancer Cell Line Encyclopedia (CCLE) dataset analyzed the expression of MEF2s in PAAD cell lines. **E**–**H** The Human Protein Atlas (HPA) dataset analyzed the expression of MEF2s in PAAD cell lines. **I**–**L** European Bioinformatics Institute (EMBL-EBI) dataset analyzed the expression of MEF2s in PAAD cell lines

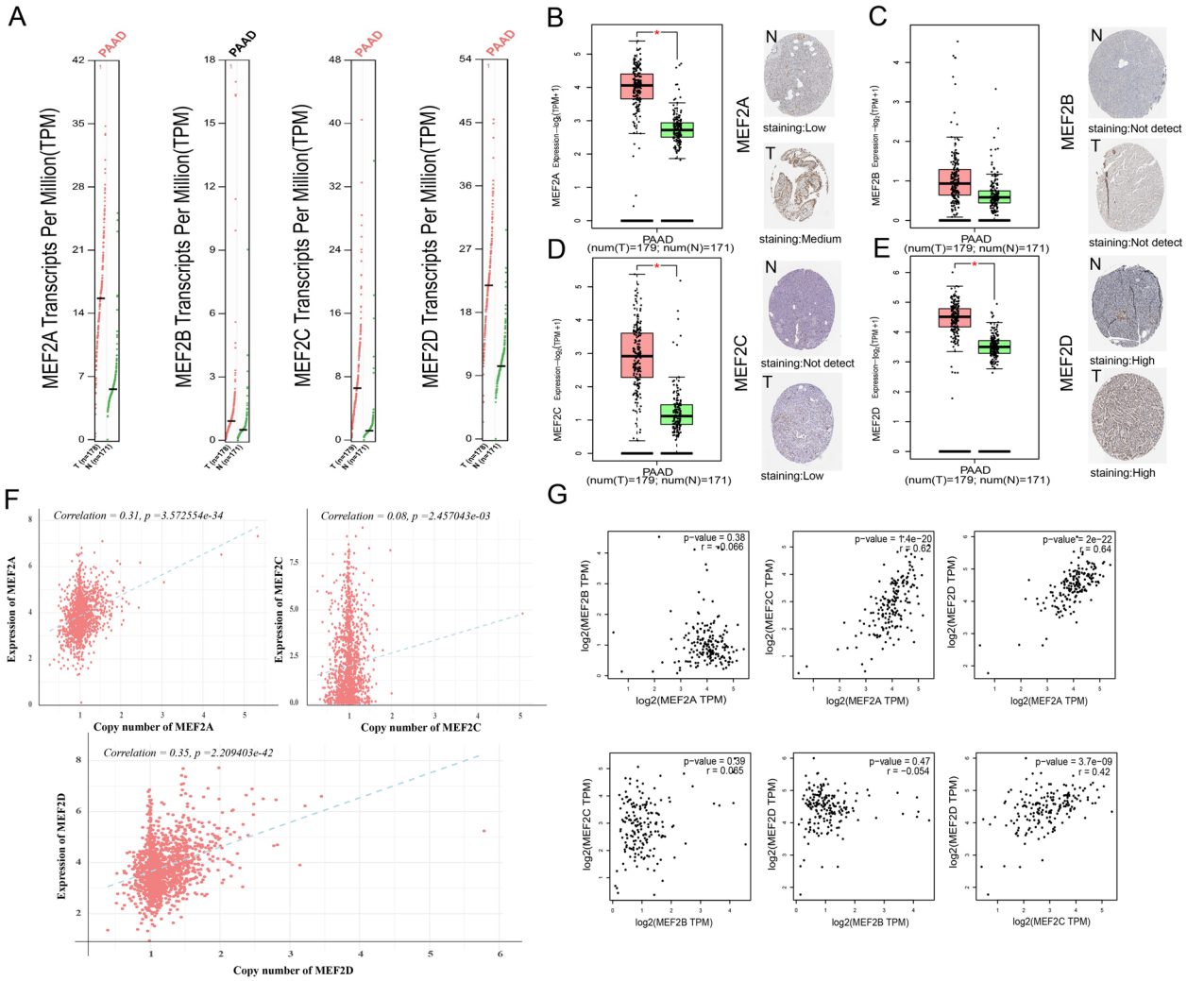


**Fig. 3** The expression of MEF2s in human cancers including PAAD patients. **A** Gene Expression Profiling Interactive Analysis (GEPIA) dataset analyzed the expression of MEF2s in PAAD. **B**-**E** GEPIA dataset analyzed the MEF2s mRNA expression between PAAD and normal pancreatic tissues. HPA dataset analyzed the MEF2s protein expression between PAAD and normal pancreatic tissues (*$P < 0.05$). **F** CCLE dataset analyzed the correction between the expression of MEF2A, MEF2C, and MEF2D and copy number variation. **G** GEPIA dataset analyzed the correction between MEF2s in PAAD

We also used the GEPIA2 database to examine the crosstalk between the MEF2s in PAAD. As shown in Fig. 3G, the expression of MEF2A was highly related to the expression of MEF2C and MEF2D. Meanwhile, MEF2C was positively correlated with MEF2D. In addition, the GEO dataset (GSE62452) confirmed that the expression of MEF2A, MEF2C, and MEF2D in tumor tissues was higher than in normal tissues (Figure S1).

### Association between tumor stages, immune subtypes, and MEF2s in PAAD patients

This study again verified the results of MEF2A, MEF2C, and MEF2D expression in normal tissues and tumor tissues, and the analysis found that MEF2A, MEF2C, and MEF2D were highly expressed in PAAD tissues (Fig. 4A). In addition, the expression level of MEF2 family was not statistically significant with the tumor stage of PAAD patients (Fig. 4B). As shown in Fig. 4C, we applied molecular typing of immune subtypes to analyze the expression of MEF2s in various immune subtypes. We noticed that the expression characteristics of MEF2C were significantly differences in diverse immune subtypes. In PAAD patients, MEF2C had the highest expression in C3 (inflammatory).

### Methylation level of MEF2s in PAAD

We found that the methylation level of MEF2s was associated with gender, Nodal Metastasis status, and TP53

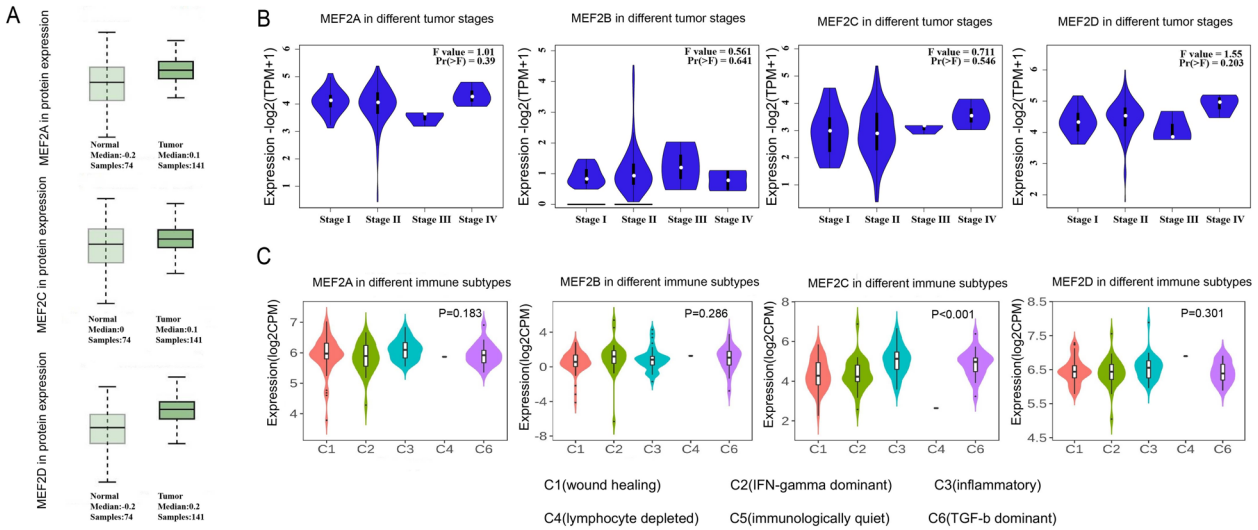


**Fig. 4** The relationship between tumor stages, immune subtypes, and MEF2s in PAAD patients. **A** MEF2A, MEF2C, and MEF2D in protein expression. **B** GEPIA dataset analyzed tumor stages of MEF2s in PAAD. **C** TISIDB dataset analyzed the expression of MEF2s in different immune subtypes. Kruskal–Wallis test evaluates the statistical significance of differential expression

mutation status in PAAD patients (Figure S2). It was found that males with PAAD had higher methylation levels than females. There was no significant difference between N0 and N1 Nodal Metastasis status in PAAD patients. In addition, there was no significant difference between TP53 mutation status and TP53 non-mutation status. Furthermore, we also used the MEXPRESS to investigate the association between gene expression and DNA methylation of MEF2s in various CPG sites. As shown in Fig. 5, negative correlations were observed between the expression level of MEF2A, MEF2C, and MEF2D and the methylation levels in multiple sites. In addition, We found that among the five CpG sites negatively associated with MEF2A expression, cg22457814 overlapped with the promoter region (Fig. 5). None of the CpG sites negatively correlated with the expression of MEF2B, MEF2C, and MEF2D had overlapping promoter regions. DiseaseMeth version 3.0 analysis displayed that the mean methylation levels of MEF2A, MEF2C, and MEF2D were all significantly reduced in PAAD compared to normal tissues ($P < 0.05$) (Figure S3).

### Prognostic value of MEF2s family members in PAAD

We then investigated the prognostic value of MEF2s in PAAD. High expression of MEF2A was related to poor OS ($p = 0.0071$) and RFS ($p = 0.0089$) of PAAD (Fig. 6A, E). High expression of MEF2B was linked to better OS ($p = 0.011$) and RFS ($p = 0.034$) of PAAD patients (Fig. 6B, F). High expression of MEF2C was associated with worse RFS of PAAD ($p = 0.043$) (Fig. 6G). However, there was no significant difference between MEF2D expression and OS ($p = 0.2$) or RFS ($p = 0.39$) of PAAD. Moreover, no relationship was detected between the expression of MEF2s and DFS of PAAD (Fig. 6I-L).

In addition, Univariate and Multivariate Cox regression analyses were used to explore whether MEF2s was a prognostic factor of PAAD independent of clinical factors such as pathological stage, age, and primary therapy outcome. The results showed that high expression of MEF2A was an independent prognostic factor for PAAD patients (HR from Univariate Cox regression analysis: 1.421 (95%CI: 1.055–1.976, $P = 0.015$); HR from Multivariate Cox regression analysis: 1.282 (95%CI: 1.017–2.082, $P = 0.024$) (Figure S4 A-B). A clinical prognostic risk score for PAAD was developed, incorporating pathological N stage, primary therapy outcome, radiation therapy, and MEF2A expression profiles (Figure S4 C). Model validation was performed through calibration curve analysis to evaluate predictive accuracy (Figure S4D). Analytical results demonstrated that the expression of MEF2A could more accurately predict the survival probability of patients. Furthermore, MEF2A expression levels exhibited a significant correlation with the prognosis of PAAD.

### Mutations of MEF2s family members in PAAD and the relationship of these mutations with survival in PAAD patients

We also analyzed the feature of MEF2s mutations in PAAD through the cBioPortal database. As presented in Fig. 7A, MEF2s were varied in 46 samples of 177 PAAD

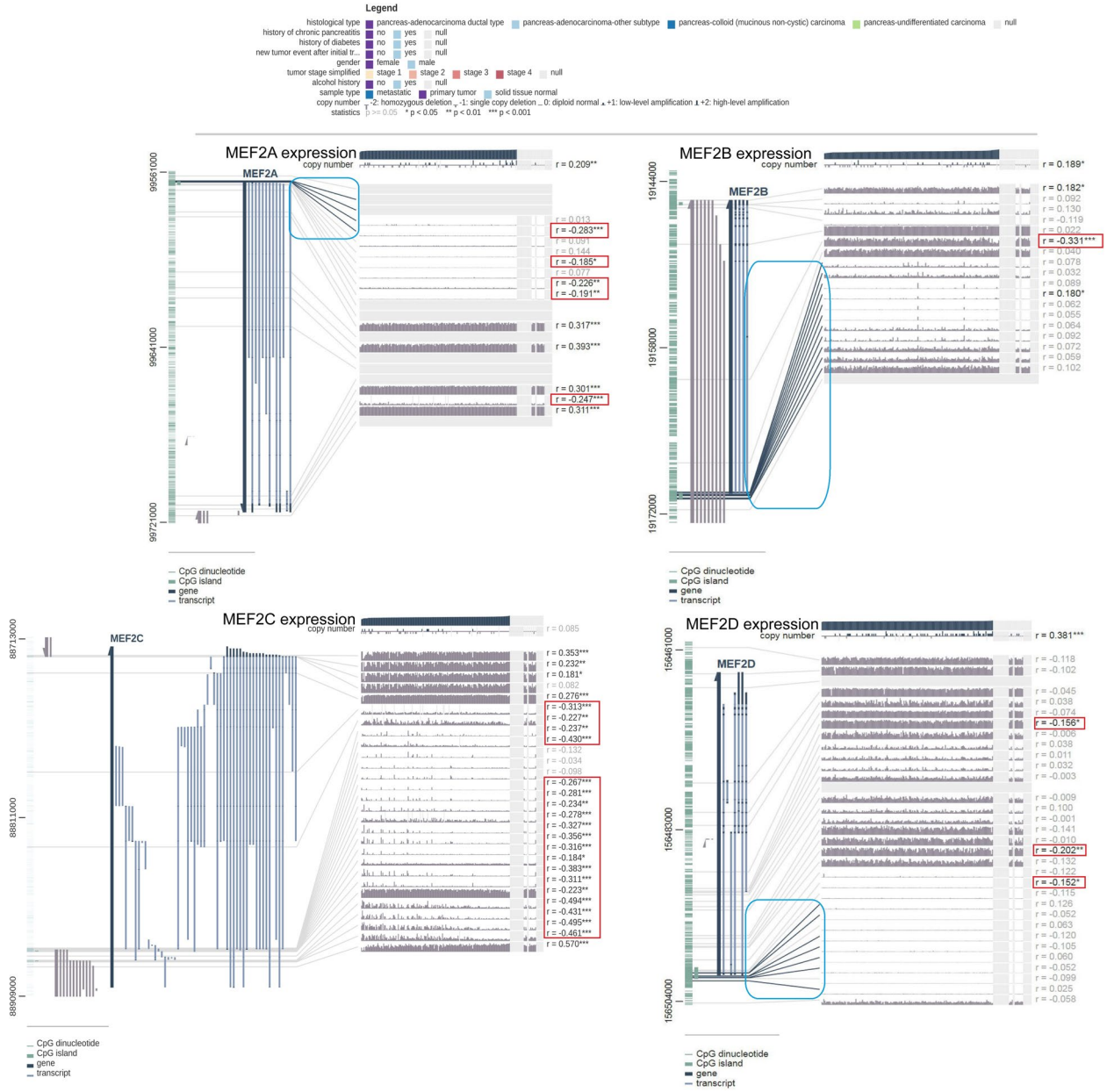


**Fig. 5** MEF2s expression and methylation status in PAAD using MEXPRESS tool. Association between MEF2s expression and DNA methylation, the Benjamini-Hochberg-adjusted *p*-value, and the Pearson correlation coefficients (r) are displayed. *$p < 0.05$, **$p < 0.01$,***$p < 0.001$

patients (26%). There was only one mutation in MEF2A, MEF2C, and MEF2D (Fig. 7B). MEF2A was a Truncating mutation, and it was shown that the "G27 Wfs* 8" mutation point was distributed in the SRF-TF domain. Both MEF2C and MEF2D were a Missense mutation. Among them, the mutation point "R256Q" of MEF2C was distributed in the second half of the domain, and the mutation point "P473L" of MEF2D was distributed at the end of the domain. In addition, no mutations were shown in MEF2B. Besides, we did not find an association between mutations in the MEF2s family members and survival in PAAD (Fig. 7C).

### Immune cells infiltration of MEF2s family members in PAAD

To explore the role of MEF2s family genes in the tumor microenvironment (TME) of PAAD, we evaluated the association between MEF2s family expression and immune cells infiltration by the TIMER database (Fig. 8A). The Spearman tests (adjusted for tumor

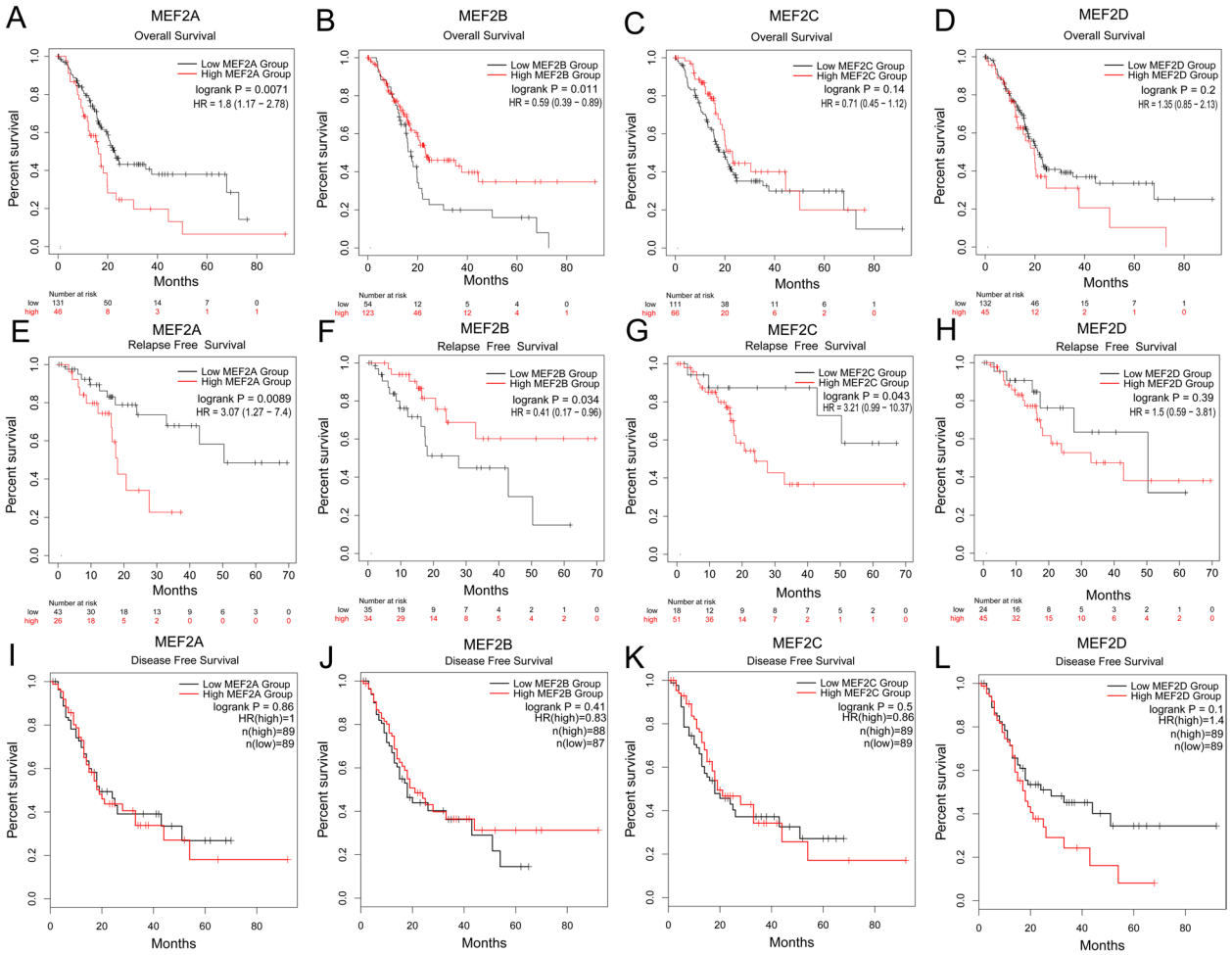


**Fig. 6** Prognostic value of MEF2s family members in PAAD. **A**-**H** Kaplan–Meier Plotter dataset analyzed the overall survival and relapse-free survival of MEF2s family members in PAAD patients. **I**-**L** GEPIA dataset analyzed the disease-free survival of MEF2s family members in PAAD patients

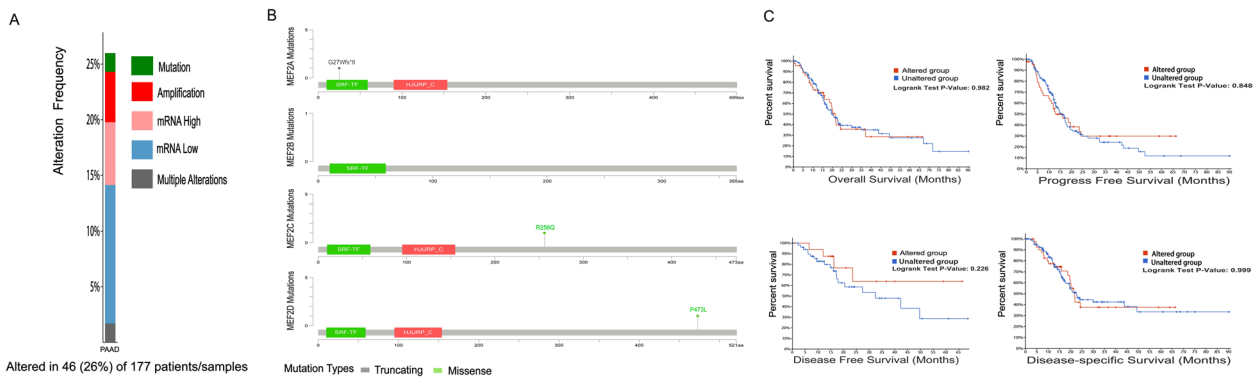


**Fig. 7** Genetic mutations in MEF2s family and its relationship with prognostic value of PAAD patients. **A**-**B** cBioPortal generated a schematic diagram of MEF2s family mutations in PAAD. **C** cBioPortal analyzed the relationship between MEF2s family gene mutations and prognostic value in PAAD

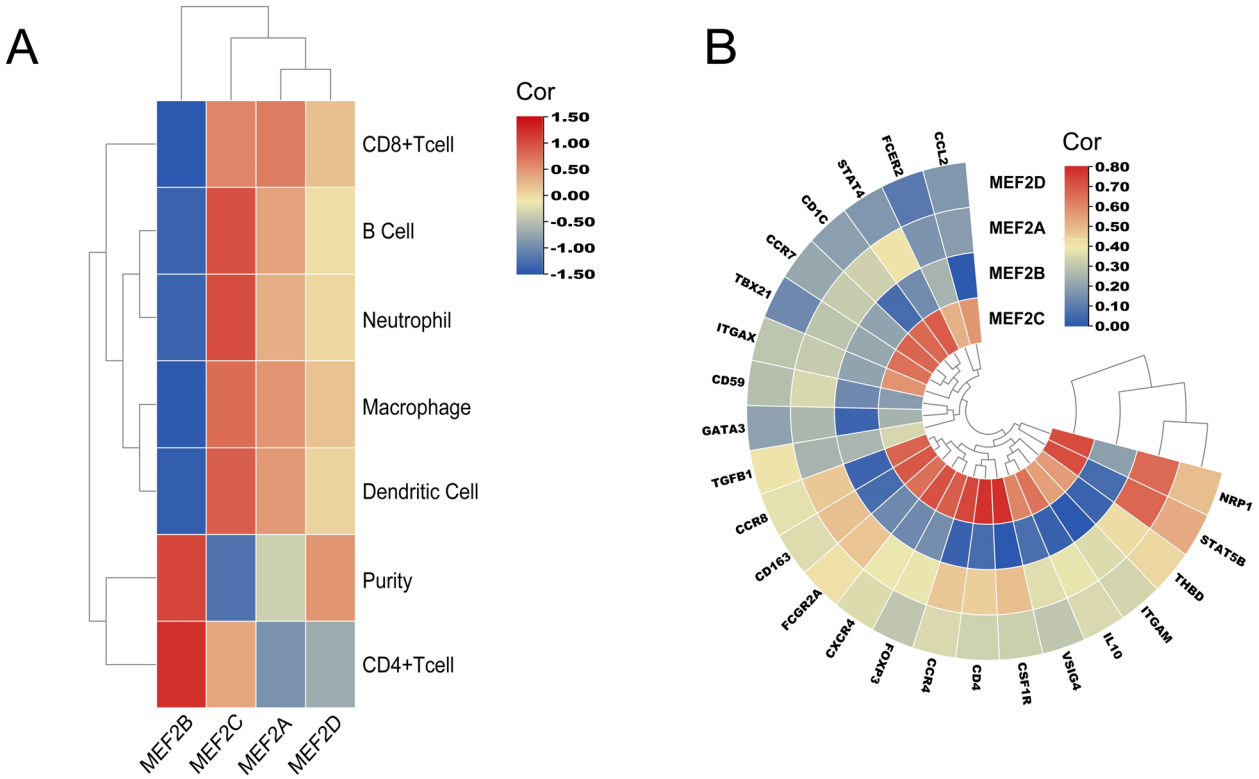


**Fig. 8** The relationship between MEF2s family and infiltrating immune cells. **A** TBtools generated a heatmap of the correlation between MEF2s and infiltrating immune cells, including CD8 + T cells, B cells, neutrophils, macrophages, Dendritic cells, and CD4 + T cells. The relevance is determined by TIMER, which only analyzes the samples in the TCGA database. **B** TBtools generated a heatmap of the correlation between MEF2s and markers of infiltrating immune cells. TIMER determines the relationship. For details, see Supplementary Table 1 of Support Information

purity) were used to investigate the coefficient of association between MEF2s expression and immune cells infiltration abundances (CD8 + T cells, B-cells, neutrophils, macrophages, dendritic cells, and CD4 + T cells) in PAAD. We noticed that MEF2A, MEF2C, and MEF2D expression positively corresponded with five immune cells infiltration (CD8 + T cells, B-cells, neutrophils, macrophages, and dendritic cells), especially for CD8 + T cells and macrophages. Interestingly, we also found a significant positive correlation between MEF2B expression and the degree of CD4 + T cells infiltration. Figure S5 shows the detailed description of all the results.

In addition, this study performs Kaplan–Meier plotter analyses of MEF2A expression in PAAD following B cells, CD4 + memory T cells, CD8 + T cells, eosinophils, macrophages, mesenchymal stem cells, natural killer T cells, regulatory T-cells. We found that high MEF2A levels in PAAD in enriched B cells ($p = 0.0069$), CD8 + T cells ($p = 0.023$), eosinophils ($p = 0.044$), macrophages ($p = 0.026$), mesenchymal stem cells ($p = 0.0089$) cohort had a worse prognosis (Figure S6). However, the high expression of MEF2B in PAAD had a better prognosis in enriched B cells ($p = 0.028$), CD4 + memory T cells ($p = 0.0023$), macrophages ($p = 0.039$), mesenchymal stem cells ($p = 0.045$), natural killer T cells ($p = 0.042$), regulatory T-cells ($p = 0.0015$) (Figure S7). High expression of MEF2C in PAAD had a better prognosis only in enriched CD4 + memory T cells ($p = 0.037$) (Figure S8). There was no significant difference between high and low MEF2D expression groups' overall survival in enriched immune cells (Figure S9).

### Correlation analysis between MEF2s expression and markers of immune infiltration

Moreover, we also studied the relationship between MEF2s and various immune infiltration markers in PAAD through the TIMER database. Six typical immune cells were included: TAMs, neutrophils, DCs, Th1 cells, Th2 cells, and Tregs. Table S1 showed the detailed description of all the results. As shown in Fig. 8B, MEF2s family members were positively correlated with immune infiltrating cell markers in PAAD. Specifically, the correlation between MEF2A expression and 21 immune infiltration markers was statistically significant ($p < 0.05$), MEF2C expression was correlated with 8 immune

infiltration markers, and MEF2D expression was related to 21 immune infiltration markers (Table S1). In addition, MEF2B was weakly connected to most markers of immune infiltration (Fig. 8B). In brief, our further analysis showed that MEF2A, MEF2C, and MEF2D played a crucial role in inhibiting the immune activity of the PAAD microenvironment.

#### Functional enrichment analysis

Using the STRING database, we analyzed the potential interactions between MEF2s and 82 neighboring genes, which were mostly correlated to MEF2s themselves (Table S2). Next, a protein–protein interactome network was created among the above genes. The results showed that CR2, PTPRC, and PIK3 CG were closely connected with MEF2s (Fig. 9A). Through the plug-in MCODE of Cytoscape, we found that the genes with the highest degree of connectivity could be divided into two parts. Some of the genes were MEF2A, MEF2B, MEF2C, and MEF2D, and the other genes were PIP4K2A, PIP5K1A, and PIK3CG. Two parts of genes were mainly related to the regulation and function of MEF2s in PAAD (Fig. 9B).

We used the Metascape database to conduct the gene ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis to speculate on the function of MEF2s and the genes that are significantly associated with MEF2s. GO enrichment analysis foresaw the functional role of target genes from the following three directions, including biological process (BP), cellular components (CC), and molecular functions (MF). As presented in Fig. 9C, biological processes analysis showed that MEF2s obviously regulated the Fc receptor signaling pathway (GO:0038093), the regulation of protein kinase activity (GO:0045859), the myeloid cell differentiation (GO:0030099), the cellular response to chemical stress (GO:0062197) in PAAD. Cellular components analysis indicated that changes were mainly concentrated in the chromosomal region (GO:0098687), the cell leading edge (GO:0031252), the nuclear chromosome (GO:0000228), the focal adhesion (GO:0005925) (Fig. 9D). Molecular functions analysis showed that the chromatin binding (GO:0003682), the phosphatidylinositol phosphate kinase activity (GO:0016307), the histone deacetylase binding (GO:0042826), the phosphotransferase activity, alcohol group as acceptor (GO:0016773) were significantly enriched in the MEF2s in PAAD (Fig. 9E).

We used the KEGG analysis to identify the pathways associated with the functions of MEF2s and 82 neighboring genes. As shown in Fig. 9F, results showed that 20 pathways were related to the functions of MEF2s alterations in PAAD. Among these pathways, hsa05140 (Leishmania infection), hsa04022 (cGMP-PKG signaling pathway), hsa05145 (Toxoplasmosis), hsa04371 (Apelin signaling pathway), and hsa04064 (NF-kappa B signaling pathway), were closely connected with the occurrence and development of PAAD (Figures S10 and S11).

## Discussion

Although previous studies found that the MEF2 gene family was dysregulated in various cancers, such as gastric cancer [35], diffuse large B cell lymphoma [13], acute myeloid leukemia [36], and colorectal cancer [37]. However, the comprehensive analysis of the role of the MEF2s in PAAD was still limited. This work explored the role of each member of MEF2s in PAAD. MEF2A, MEF2C, and MEF2D were found to be highly expressed in PAAD patients' tissues compared to normal tissues. Besides, negative correlations were observed between the expression level of MEF2A, MEF2C, and MEF2D and the methylation levels in multiple sites. The mean methylation levels of MEF2A, MEF2C, and MEF2D were all significantly reduced in PAAD compared to normal tissues. High expression of MEF2A was correlated with poor OS and RFS in PAAD. High expression of MEF2C was associated with worse RFS of PAAD. In addition, we noticed that the expression of MEF2A, MEF2C, and MEF2D notably positively corresponded to the level of CD8 + T cells and macrophages infiltration, as well as a series of gene markers of specific immune cells. We also noticed that the function of MEF2s and the genes significantly associated with MEF2s affected BP such as Fc receptor signaling pathway (GO:0038093), CC such as chromosomal region (GO:0098687), MF such as chromatin binding (GO:0003682), KEGG pathway such as Leishmania infection (hsa05140). Our study provided a basis for the in-depth understanding of the heterogeneity and complexity of the molecular biological characteristics of PAAD.

According to previous reports, overexpression of MEF2A can simultaneously activate EMT-related TF and Wnt/β-catenin signaling pathways, induce the epithelial-mesenchymal transition (EMT), and increase the frequency of cancer formation and metastasis [35]. Driven by MEF2A-regulated enhancers, lncRNA BDNF-AS activates the RNH1/TRIM21/mTOR cascade, thereby inducing the malignant progression of breast cancer [36]. We found that a high MEF2A expression in PAAD patients' tissues compared to normal tissues. Then we investigated the methylation level of MEF2A in PAAD, and we found that its DNA methylation level was negatively correlated with its expression levels. Meanwhile, methylation levels of MEF2A were significantly reduced in PAAD compared

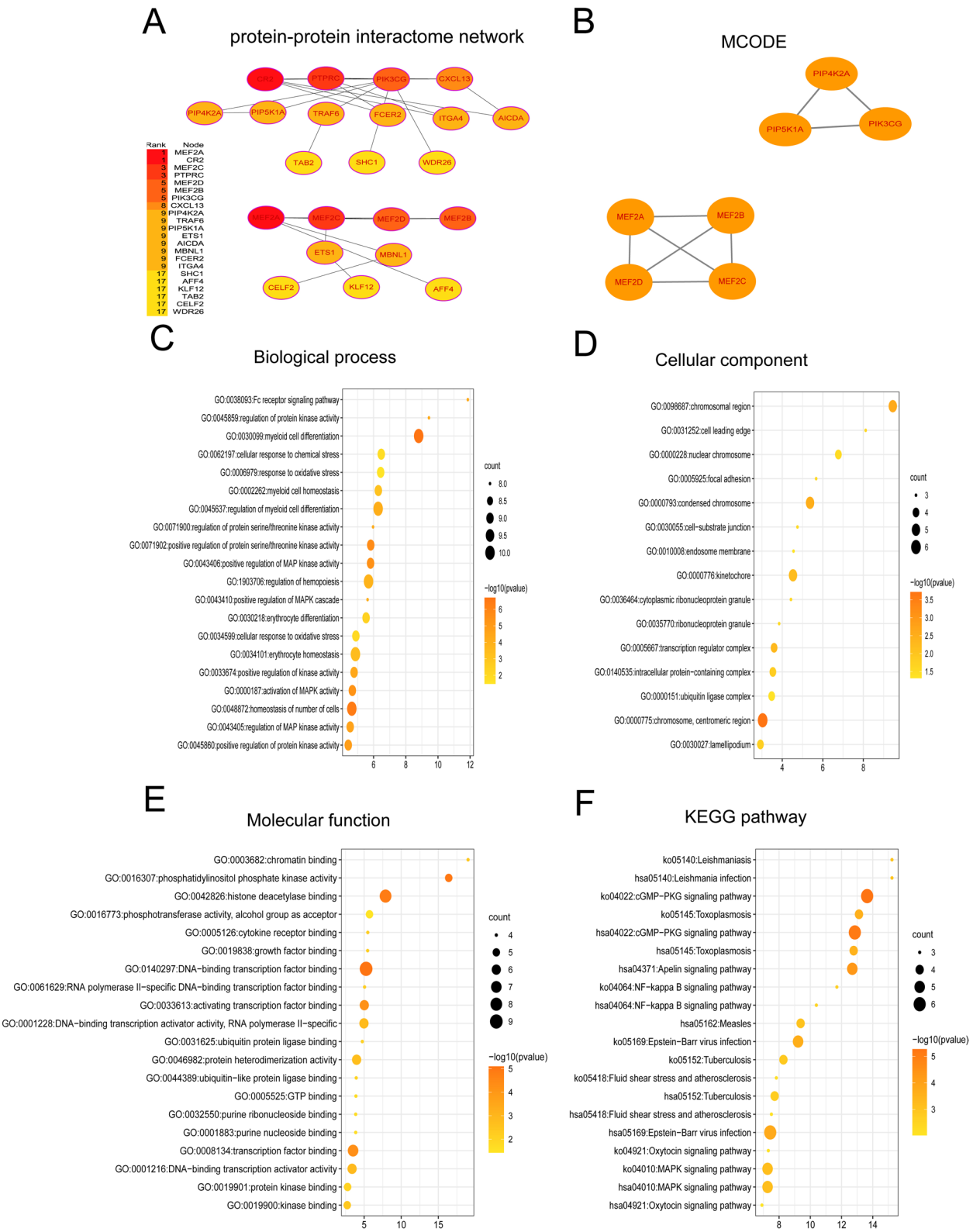


**Fig. 9** Enrichment analysis of MEF2s in patients with PAAD. **A** Cytoscape generated protein–protein interaction (PPI) network for MEF2s. **B** Cytoscape generated the most connected genes in the two-part PPI network for MEF2s. **C** Biological processes. **D** Cellular component. **E** Molecular functions. **F** KEGG pathway analysis

to normal tissues. More importantly, high expression of MEF2A was correlated with poor OS and RFS in PAAD. Multivariate analysis showed that MEF2A expression was an independent prognostic risk factor for PAAD. We discovered that MEF2A expression was significantly associated with five immune cells infiltration, including CD8 + T cells and macrophages, and immune infiltration markers, suggesting that MEF2A could reflect the immune status and play a vital role in tumor immune regulation. These findings suggested the potential of MEF2A as a prognostic biomarker.

So far, the study of the expression role of MEF2B in PAAD is limited. From the perspective of phylogeny and sequence homology, MEF2B is the furthest member of the family [37]. Among the members of the MEF2s family, MEF2B is the most divergent and receives the least attention, which is caused by the difficulty of producing MEF2B specific reagents [38]. In our report, MEF2B was not expressed in PAAD patients. In addition, MEF2B mutation was not correlated with survival. Furthermore, MEF2B was weakly connected to immune infiltration. The research results suggested that the function of MEF2B differed from that of other MEF2 family members. The reasons may be as follows: First, MEF2B stood out distantly from the remaining three MEF2 branches in vertebrates, primarily due to the absence of the HJURP_C (Holliday junction recognition protein C-terminal) region. Second, the ratio of non-synonymous to synonymous nucleotide substitution rates indicated that MEF2B undergoes more rapid evolution compared to the other three MEF2 proteins, even though all four branches were subject to purifying selection. Third, a pair model of M0 versus M3 revealed that variable selection exists among MEF2 proteins, while branch-site analysis indicated that positions 53 and 64 on the MEF2B branch were subject to positive selection [39].

There are multiple pieces of evidence that MEF2C is involved in tumor progression [37]. In fact, MEF2C promotes the metastasis and development of PAAD by inducing the transcription of metalloproteinase (MMP) 10 [40]. Data indicated that in the process of disease progression, its up-regulation in colorectal cancer and its relationship with breast cancer invasion support the carcinogenic function of MEF2C [41, 42]. We noticed that MEF2C expression was higher in PAAD tissues compared to normal tissues. In addition, we found that MEF2C expression was related to different immune subtypes. High expression of MEF2C was associated with worse RFS of PAAD, but not significantly associated with DFS. This result might reflect the different focus of RFS and DFS in evaluating the effectiveness of PAAD treatment. RFS includes local and distant recurrence, contralateral cancer, and death without recurrence [43]. DFS refers to the time from the end of treatment to any disease-related event, including recurrence, metastasis, a second primary tumor, or death. DFS had a broader range of events and may mask the association of specific mechanisms (such as relapse) with markers. In addition, RFS is more suitable for assessing the risk of recurrence after local treatment, reflecting the biological behavior of the tumor microenvironment or residual lesions [43]. DFS is often used to evaluate the overall efficacy of systemic therapies. In PAAD, RFS might be associated with local recurrence and distant metastasis as events, while DFS might be affected by systemic metastasis or complications. Therefore, MEF2C as a biomarker for the risk of local recurrence might exhibit a significant correlation in RFS, whereas it could be obscured by other factors of disease progression in DFS. Furthermore, we also noticed that MEF2C expression was significantly correlated with immune infiltration. These results indicated that MEF2C might be a potential oncogene of PAAD.

Studies found that MEF2D is the most famous member of the MEF2s family genes because it influences the development of human cancer [44]. Malignant tumors of the hematological system are the initial reports of MEF2D in human tumors [45, 46]. In addition, MEF2D has been reported as a potential therapeutic target for patients with the following tumors: primary liver cancer [16], gallbladder cancer [15], colorectal cancer [47], and ovarian cancer [14]. In our report, we observed that MEF2D expression in PAAD tissues was higher than that in normal pancreatic tissues, similar to the results of Song et al.' s [48] studies. Moreover, MEF2D expression was negatively associated with DNA methylation. However, this study had not yet discovered the relationship between MEF2D expression and survival. The lack of prognostic significance of MEF2D did not rule out its biological significance, as it might be involved in processes such as the cGMP-PKG signaling pathway and the Toxoplasmosis signaling pathway that were critical to PAAD progression, but these effects were not directly reflected in survival outcomes. Studies found that MEF2D leads to PAAD through the AKT/GSK- 3β signaling pathway [48]. MEF2D mutation was not associated with survival in PAAD. Similar results were found in the study by Li et al. [49] Besides, we noticed that MEF2D expression was connected with immune infiltration, showing that MEF2D affected suppressing the immune activity of PAAD. Thus, MEF2D had potential biological significance.

This study found that high expression of the MEF2s family was associated with poor survival in PAAD, such as MEF2A and MEF2C. Clocchiatti A et al. [50] found that Class IIa HDACs repressive activities on MEF2-depedent transcription are associated with poor

prognosis of ER breast tumors +. Di et al. [51] also showed that the combined effect of high MEF2 and class IIa HDACs levels is detrimental to the survival of cancer patients. The reason may be that UPS-mediated degradation can remove MEF2 from promoters and enhancers, but their conversion to repressors can provide the strongest silencing, resulting in a worse prognosis. However, this study also observed that MEF2s family expression did not vary significantly or was low according to the stage. This phenomenon may suggest that the role of the MEF2s family in tumor progression may involve other biological processes. Studies showed that MEF2 acted as an oncogene in immature T-cell acute lymphoblastic leukemia, B-cell acute lymphoblastic leukemia and hepatocellular carcinoma by regulating various processes such as proliferation, apoptosis, or epithelial-mesenchymal transition [52–54]. Additionally, Xia et al. [55] found that MTOR (mechanistic target of rapamycin kinase) complex 1 (MTORC1) activation mediated by MEF2s was particularly relevant to PAAD.

Modifications to the methylation status within promoter regions play a pivotal role in regulating gene expression, with an increase in methylation typically leading to transcriptional suppression, while a decrease in methylation generally results in transcriptional enhancement [56]. Furthermore, methylated could attract proteins that bind to methyl-CpG sites, such as MBD2 and MeCP2, which in turn activate histone deacetylases (HDACs) and additional chromatin-modifying complexes [57]. Studies also indicated that subtle changes in methylation could trigger widespread alterations in histone modification patterns, including the loss of active chromatin markers such as H3 K9ac and H3 K4 me3, and the establishment of repressive markers like H3 K27 me3, thereby reinforcing a repressive chromatin environment [58]. Additionally, even a slight reduction in promoter methylation, affecting only a few CpG sites, can result in gene silencing, a phenomenon particularly relevant in cancer-associated genes [59]. Minor methylation alterations in promoter regions can induce changes in histone modifications, significantly impacting gene expression [60]. Therefore, nuanced methylation modifications in promoter regions were not isolated events but acted as catalysts in the process of gene regulation by obstructing transcription factor access, inducing repressive complexes, and altering chromatin architecture.

This study comprehensively explored the role of each member of MEF2s in PAAD. In the current study, we analyzed the multimolecular mechanisms of MEF2s in PAAD at the level of expression, methylation, survival, mutation, immune infiltration, and related gene enrichment analysis. These were the strengths of our study, but this study also had the following limitations. Although the research combined multiple databases, most datasets were retrospective, so we needed to conduct prospective research. In addition, multiple datasets were combined, leading to heterogeneity in the research background, so we needed a larger sample size.

In conclusion, this study found the overexpression of MEF2A, MEF2C, and MEF2D in patients with PAAD. High expression of MEF2A was correlated with poor OS and RFS in PAAD. High expression of MEF2C was associated with worse RFS of PAAD. Besides, negative correlations were observed between the expression level of MEF2A, MEF2C, and MEF2D and the methylation levels in multiple sites. This study also noticed that the expression of MEF2A, MEF2C, and MEF2D notably corresponded to immune cells. Our study provides a basis for the in-depth understanding of the heterogeneity and complexity of the molecular biological characteristics of PAAD. Meanwhile, it is suggested that MEF2A could serve as a prognostic biomarker for PAAD, MEF2C might function as a potential oncogene for PAAD, and MEF2D had potential biological significance.

**Abbreviations**

| | |
|---|---|
| PAAD | Pancreatic adenocarcinoma |
| MEF2 | Myocyte enhancer factor-2 |
| CCLE | Cancer Cell Line Encyclopedia |
| HPA | Human Protein Atlas |
| EMBL-EBI | European Bioinformatics Institute |
| GEPIA | Gene Expression Profiling Interactive Analysis |
| TISIDB | Tumor Immune System Interactions Database |
| OS | Overall survival |
| RFS | Relapse-free survival |
| DFS | Disease-free survival |
| HR | Hazard ratio |
| CI | Confidence intervals |
| CBioPortal | CBio Cancer Genomics Portal |
| TIMER | Tumor Immune Estimation Resource |
| CNV | Copy number variation |
| TME | tumor microenvironment |
| PPI | Protein-protein interactome |
| GO | Gene ontology |
| KEGG | Kyoto Encyclopedia of Genes and Genomes |
| BP | Biological process |
| CC | cellular components |
| MF | Molecular functions |
| EMT | Epithelial-mesenchymal transition |
| MMP | Metalloproteinase |
| TCGA | The Cancer Genome Atlas |
| GTEx | Genotype-Tissue Expression |
| MCODE | Molecular Complex Detection |
| MTORC1 | Mechanistic target of rapamycin kinase complex 1 |

## Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12885-025-14107-x.

Supplementary Material 1: Figure S1. The expression of MEF2s is different between normal tissues and PAAD tissues

Supplementary Material 2: Figure S2. The promoter methylation level of MEF2s in PAAD through the UALCAN database. (A) patients's gender. (B) nodal metastasis status. (C) TP53 mutation status

Supplementary Material 3: Figure S3. The methylation levels of MEF2s in PAAD tissues and normal tissues were examined using DiseaseMeth 3.0 database

Supplementary Material 4: Figure S4. Determination of independent risk factors and construction of rograms. (A) Univariate Cox regression of MEF2s expression in PAAD patients with overall survival. (B) Multivariate Cox regression of MEF2A expression in PAAD patients with overall survival. (C) The nomogram can predict the 1-year, 2-year, and 3-year overall survival of PAAD patients. (D) Nomogram calibration to predict 1-, 2- and 3-year survival probabilities. The gray line represents actual survival

Supplementary Material 5: Figure S5. The association between MEF2s expression and immune cells infiltration(B-cells, CD8+ T cells, CD4+ T cells, macrophages, neutrophils, and dendritic cells) in PAAD by the TIMER database

Supplementary Material 6: Figure S6. Kaplan-Meier plotter analyses of MEF2A expression in PAAD following immune cells

Supplementary Material 7: Figure S7. Kaplan-Meier plotter analyses of MEF2B expression in PAAD following immune cells

Supplementary Material 8: Figure S8. Kaplan-Meier plotter analyses of MEF2C expression in PAAD following immune cells

Supplementary Material 9: Figure S9. Kaplan-Meier plotter analyses of MEF2D expression in PAAD following immune cells

Supplementary Material 10: Figure S10. cGMP-PKG signaling pathway regulated by the MEF2s Alteration in PAAD

Supplementary Material 11: Figure S11. TOXOPLASMOSIS signaling pathway regulated by the MEF2s Alteration in PAAD

Supplementary Material 12: Table S1. Correlation analysis between MEF2s and Gene Markers of Immune Cells in TIMER

Supplementary Material 13: Table S2. The 82 neighboring genes, which were mostly correlated to MEF2s themselves

**Acknowledgements**
Not applicable.

**Authors' contributions**
C-XZ: Conceptualization, Data curation, Formal analysis, Methodology, Software, Visualization, Writing an original draft. X-RD and L-PM: Methodology, Software, Visualization. X-RD, L-PM, A-QH, and YG: Conceptualization, Data curation, Validation, Software, Visualization. A-QH and YG: Data curation, Supervision. FY and L-PM: Software, Writing review & editing. YD: Conceptualization, Methodology, Writing review & editing. All authors approved the final draft of the manuscript. All authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The corresponding author attests that all listed authors meet authorship criteria and that no others meeting the criteria have been omitted.

**Funding**
This work was supported by grants from the Jiangsu Province 2023 Preventive Medicine Topic Guidance Project (Y12023023), Mandatory general project of Nantong Health Commission (MS2023088), and Jiangsu Province occupational health research project (JSZJ20241103).

**Data availability**
The databases used in this research are available in online repositories. These data come from here: https://www.broadinstitute.org/ccle, https://www.proteinatlas.org/, https://www.ebi.ac.uk, http://gepia.cancer-pku.cn/, http://ualcan.path.uab.edu, http://cis.hku.hk/TISIDB/index.php, https://www.ncbi.nlm.nih.gov/geo/, https://mexpress.ugent.be/, http://diseasemeth.edbc.org/, http://www.kmplot.com, http://www.cbioportal.org/, https://cistrome.shinyapps.io/timer/, http://string-db.org/, http://www.metascape.org/.

## Declarations

**Ethics approval and consent to participate**
The study was conducted based on data in the public databases, there is no ethical statement to be declared.

**Consent for publication**
Not applicable.

**Competing interests**
The authors declare no competing interests.

**Author details**
[1]Department of Public Health, Affiliated Nantong Hospital of Shanghai University (The Sixth People's Hospital of Nantong), Nantong, Jiangsu 226011, China. [2]Department of Oncology, Affiliated Nantong Hospital of Shanghai University (The Sixth People's Hospital of Nantong), Nantong, Jiangsu 226011, China.

Received: 19 December 2024 Accepted: 8 April 2025
Published online: 25 April 2025

**References**

1. Organization WH. GLOBOCAN: estimated cancer incidence, mortality and prevalence worldwide in 2018. 2019.
2. Rahib L, Smith BD, Aizenberg R, Rosenzweig AB, Fleshman JM, Matrisian LM. Projecting cancer incidence and deaths to 2030: the unexpected burden of thyroid, liver, and pancreas cancers in the United States. Cancer Res. 2014;74(14):2913–21.
3. Ko J, Bhagwat N, Black T, Yee SS, Na YJ, Fisher S, Kim J, Carpenter EL, Stanger BZ, Issadore D. miRNA profiling of magnetic nanopore-isolated extracellular vesicles for the diagnosis of pancreatic cancer. Cancer Res. 2018;78(13):3688–97.
4. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2017. CA Cancer J Clin. 2015;60(1):277–300.
5. Hoos WA, et al. Pancreatic cancer clinical trials and accrual in the United States. J Clin Oncol. 2013;31(27):3432.
6. Vincent A, Herman J, Schulick R, Hruban RH, Goggins M. Pancreatic cancer. Lancet. 2011;378(9791):607–20.
7. Molkentin JD, Olson EN. Combinatorial control of muscle development by basic helix-loop-helix and MADS-box transcription factors. Proc Natl Acad Sci. 1996;93(18):9366–73.
8. Mckinsey TA, Zhang CL, Olson EN. MEF2: a calcium-dependent regulator of cell division, differentiation and death. Trends Biochem Sci. 2002;27(1):40–7.
9. Lin Q, Schwarz J, Bucana C, Olson EN. Control of mouse cardiac morphogenesis and myogenesis by transcription factor MEF2C. Science. 1997;276(5317):1404–7.
10. Hayashi M, Kim SW, Imanaka-Yoshida K, Yoshida T, Lee JD. Targeted deletion of BMK1/ERK5 in adult mice perturbs vascular integrity and leads to endothelial failure. J Clin Invest. 2004;113(8):1138–48.
11. Arnold MA, Kim Y, Czubryt MP, Phan D, Olson EN. MEF2C transcription factor controls chondrocyte hypertrophy and bone development. Dev Cell. 2007;12(3):377–89.
12. Potthoff MJ, Olson EN. MEF2: a central regulator of diverse developmental programs. Development. 2007;134(23):4131–40.
13. Ying CY, Dominguez-Sola D, Fabi M, et al. MEF2B mutations lead to deregulated expression of the oncogene BCL6 in diffuse large B cell lymphoma. Nat Immunol. 2013;14(10):1084.
14. Li X, Zhang Y, Chai X, Zhou S, Tao G. Overexpression of MEF2D contributes to oncogenic malignancy and chemotherapeutic resistance in ovarian carcinoma. Am J Cancer Res. 2019;9(5):887–905.
15. Wang W, Chen LC, Qian JY, Zhang Q. MiR-335 promotes cell proliferation by inhibiting MEF2D and sensitizes cells to 5-Fu treatment in gallbladder carcinoma. Eur Rev Med Pharmacol Sci. 2019;23(22):9829–39.
16. Ma L, Liu J, Liu L, et al. Overexpression of the transcription factor MEF2D in hepatocellular carcinoma sustains malignant character by suppressing G2-M transition genes. Cancer Res. 2014;74(5):1452.

17. Uhlén M, Björling E, Agaton C, et al. A human protein atlas for normal and cancer tissues based on antibody proteomics. Mol Cell Proteomics. 2005;4(12):1920–32.
18. Barretina J, Caponigro G, Stransky N, Venkatesan K, Al E. The cancer cell line encyclopedia enables predictive modelling of anticancer drug sensitivity. Nature. 2012;483(7391):603.
19. Li W, Andrew C, Mahmut U, et al. The EMBL-EBI bioinformatics web and programmatic tools framework. Nucleic Acids Res. 2015;W1:580–4.
20. Tang Z, Li C, Kang B, Gao G, Li C, Zhang Z. GEPIA: a web server for cancer and normal gene expression profiling and interactive analyses. Nucleic Acids Res. 2017;W1:W1.
21. Logsdon CD, Simeone DM, Binkley C, et al. Molecular profiling of pancreatic adenocarcinoma and chronic pancreatitis identifies multiple genes differentially regulated in pancreatic cancer. Cancer Res. 2003;63(10):2649–57.
22. Badea L, Herlea V, Dima SO, Dumitrascu T, Popescu I. Combined gene expression analysis of whole-tissue and microdissected pancreatic ductal adenocarcinoma identifies genes specifically overexpressed in tumor epithelia-the authors reported a combined gene expression analysis of whole-tissue and microdissected pancreatic ductal adenocarcinoma identifies genes specifically overexpressed in tumor epithelia. Hepatogastroenterology. 2008;55(88):2016.
23. Chandrashekar DS, Bashel B, Balasubramanya SAH, Creighton CJ, Varambally S. UALCAN: a portal for facilitating tumor subgroup gene expression and survival analyses. Neoplasia. 2017;19(8):649–58.
24. Ru B, Ngar WC, Tong Y, et al. TISIDB: an integrated repository portal for tumor–immune system interactions. Bioinformatics. 2019;20:20.
25. Koch A, De MT, Jeschke J, Van CW. MEXPRESS: visualizing expression, DNA methylation and clinical TCGA data. BMC Genomics. 2015;16:636.
26. Xing J, Zhai R, Wang C, Liu H, Zeng J, Zhou D, Zhang M, Wang L, Wu Q, Gu Y, Zhang Y. DiseaseMeth version 3.0: a major expansion and update of the human disease methylation database. Nucleic Acids Res. 2022;50(D1):D1208–15.
27. Menyhárt O, Nagy Á, Győrffy B. Determining consistent prognostic biomarkers of overall survival and vascular invasion in hepatocellular carcinoma. R Soc Open Sci. 2018;5(12):181006.
28. Gyorffy B, Surowiak P, Budczies J, Lanczky A. Online survival analysis software to assess the prognostic value of biomarkers using transcriptomic data in non-small-cell lung cancer. PLoS One. 2013;8:e82241.
29. Cerami E, Gao J, Dogrusoz U, Gross BE, Schultz N. The cBio cancer genomics portal: an open platform for exploring multidimensional cancer genomics data. Cancer Discov. 2012;2(5):401–4.
30. Gao J, Aksoy BA, Dogrusoz U, Dresdner G, Gross B, Sumer SO, Sun Y, Jacobsen A, Sinha R, Larsson E, Cerami E, Sander C, Schultz N. Integrative analysis of complex cancer genomics and clinical profiles using the cBioPortal. Sci Signal. 2013;6(269):pl1.
31. Li T, Fan J, Wang B, et al. TIMER: a web server for comprehensive analysis of tumor-infiltrating immune cells. Cancer Res. 2017;77(21):e108.
32. Chen C, Rui X, Hao C, He Y. TBtools, a Toolkit for Biologists integrating various HTS-data handling tools with a user-friendly interface. Cold Spring Harbor Laboratory. 2018(8).
33. von Mering C, Huynen M, Jaeggi D, Schmidt S, Bork P, Snel B. STRING: a database of predicted functional associations between proteins. Nucleic Acids Res. 2003;31(1):258–61.
34. Zhou Y, Zhou B, Pache L, Chang M, Khodabakhshi AH, Tanaseichuk O, Benner C, Chanda SK. Metascape provides a biologist-oriented resource for the analysis of systems-level datasets. Nat Commun. 2019;10(1):1523.
35. Xiao Q, Gan Y, Li Y, et al. MEF2A transcriptionally upregulates the expression of ZEB2 and CTNNB1 in colorectal cancer to promote tumor progression. Oncogene. 2021;40(19):3364–77.
36. Lin X, Dinglin X, Cao S, Zheng S, Wu C, Chen W, Li Q, Hu Q, Zheng F, Wu Z, Lin DC, Yao Y, Xu X, Xie Z, Liu Q, Yao H, Hu H. Enhancer-driven lncRNA BDNF-AS induces endocrine resistance and malignant progression of breast cancer through the RNH1/TRIM21/mTOR cascade. Cell Rep. 2020;31(10):107753.
37. Di Giorgio E, Hancock WW, Brancolini C. MEF2 and the tumorigenic process, hic sunt leones. Biochim Biophys Acta Rev Cancer. 2018;1870(2):261–73.
38. Pon JR, Marra MA. MEF2 transcription factors: developmental regulators and emerging cancer genes. Oncotarget. 2016;7(3):2297–312.
39. Wu W, de Folter S, Shen X, Zhang W, Tao S. Vertebrate paralogous MEF2 genes: origin, conservation, and evolution. PLoS One. 2011;6(3):e17334.
40. Zhang JJ, Zhu Y, Xie KL, et al. Yin Yang-1 suppresses invasion and metastasis of pancreatic ductal adenocarcinoma by downregulating MMP10 in a MUC4/ErbB2/p38/MEF2C-dependent mechanism. Mol Cancer. 2014;13(1):130–130.
41. Schuetz CS, Bonin M, Clare SE, et al. Progression-specific genes identified by expression profiling of matched ductal carcinomas in situ and invasive breast tumors, combining laser capture microdissection and oligonucleotide microarray analysis. Cancer Res. 2006;66(10):5278–86.
42. Chen G, Han N, Li G, et al. Time course analysis based on gene expression profile and identification of target molecules for colorectal cancer. Cancer Cell Int. 2016;16(1):22.
43. Burstein HJ, Prestrud AA, Seidenfeld J, Anderson H, Buchholz TA, Davidson NE, Gelmon KE, Giordano SH, Hudis CA, Malin J, Mamounas EP, Rowden D, Solky AJ, Sowers MR, Stearns V, Winer EP, Somerfield MR, Griggs JJ, American Society of Clinical Oncology. American Society of Clinical Oncology clinical practice guideline: update on adjuvant endocrine therapy for women with hormone receptor-positive breast cancer. J Clin Oncol. 2010;28(23):3784–96.
44. Kong J, Liu X, Li X, et al. Pokemon promotes the invasiveness of hepatocellular carcinoma by enhancing MEF2D transcription. Hepatol Int. 2016;10(3):493–500.
45 Prima V, Gore L, Caires A, et al. Cloning and functional characterization of MEF2D/DAZAP1 and DAZAP1/MEF2D fusion proteins created by a variant t(1;19)(q23;p13.3) in acute lymphoblastic leukemia. Leukemia. 2005;19(5):806–13.
46. Prima V, Hunger SP. Cooperative transformation by MEF2D|[sol]|DAZAP1 and DAZAP1|[sol]|MEF2D fusion proteins generated by the variant t(1;19) in acute lymphoblastic leukemia. Leukemia. 2007;21(12):2470–5.
47. Su L, Luo Y, et al. MEF2D transduces microenvironment stimuli to ZEB1 to promote epithelial-mesenchymal transition and metastasis in colorectal cancer. Cancer Res. 2016;76(17):5054.
48. Song Z, Chan F, Lu Y, Yong G, Dong C. Overexpression and biological function of MEF2D in human pancreatic cancer. Am J Transl Res. 2017;9(11):4836–47.
49. Li Y, Zhang L, Nong J, Bian S, Zhao Z, Ren Y, Lin X, Bian X. Expression of MEF2D on nasopharyngeal carcinoma tissues and its influence of prognostic. Lin Chuang Er Bi Yan Hou Tou Jing Wai Ke Za Zhi. 2011;25(18):840–2, 847 Chinese.
50. Clocchiatti A, Di Giorgio E, Ingrao S, Meyer-Almes FJ, Tripodo C, Brancolini C. Class IIa HDACs repressive activities on MEF2-depedent transcription are associated with poor prognosis of ER$^{+}$breast tumors. FASEB J. 2013;27(3):942–54.
51. Di Giorgio E, Franforte E, Cefalù S, Rossi S, Dei Tos AP, Brenca M, Polano M, Maestro R, Paluvai H, Picco R, Brancolini C. The co-existence of transcriptional activator and transcriptional repressor MEF2 complexes influences tumor aggressiveness. PLoS Genet. 2017;13(4):e1006752.
52. Yu W, Huang C, Wang Q, et al. MEF2 transcription factors promotes EMT and invasiveness of hepatocellular carcinoma through TGF-β1 autoregulation circuitry. Tumor Biol. 2014;35(11):10943–51.
53. McDonald C, Karstegl CE, Kellam P, et al. Regulation of the Epstein-Barr virus zp promoter in B lymphocytes during reactivation from latency. J Gen Virol. 2010;91(3):622–9.
54. Nagel S, Meyer C, Quentmeier H, et al. MEF2C is activated by multiple mechanisms in a subset of T-acute lymphoblastic leukemia cell lines. Leukemia. 2008;22(3):600–7.
55. Xia L, Nie T, Lu F, Huang L, Shi X, Ren D, Lu J, Li X, Xu T, Cui B, Wang Q, Gao G, Yang Q. Direct regulation of FNIP1 and FNIP2 by MEF2 sustains MTORC1 activation and tumor progression in pancreatic cancer. Autophagy. 2024;20(3):505–24.
56. Kaaij LJ, Mokry M, Zhou M, Musheev M, Geeven G, Melquiond AS, de Jesus Domingues AM, de Laat W, Niehrs C, Smith AD, Ketting RF. Enhancers reside in a unique epigenetic environment during early zebrafish development. Genome Biol. 2016;17(1):146.

57. Nan X, Ng HH, Johnson CA, Laherty CD, Turner BM, Eisenman RN, Bird A. Transcriptional repression by the methyl-CpG-binding protein MeCP2 involves a histone deacetylase complex. Nature. 1998;393(6683):386–9.
58. Rountree MR, Bachman KE, Herman JG, Baylin SB. DNA methylation, chromatin inheritance, and cancer. Oncogene. 2001;20(24):3156–65.
59. Kondo Y, Issa JP. Epigenetic changes in colorectal cancer. Cancer Metastasis Rev. 2004;23(1–2):29–39.
60. Fahrner JA, Eguchi S, Herman JG, Baylin SB. Dependence of histone modifications and gene expression on DNA hypermethylation in cancer. Cancer Res. 2002;62(24):7213–8.

## Publisher's Note